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TGF-β in Th17 Cell Development: The Truth Is Out There

Robin D. Hatton[1,*]
[1]Department of Pathology, University of Alabama, Birmingham, AL 35294, USA
*Correspondence: rdzialo@uab.edu
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As Th17 cell developmental requirements continue to be studied, Gutcher et al. (2011) demonstrate in this issue of *Immunity* that autocrine TGF-β cytokine promotes Th17 cell development and maintenance.

The initial description of T helper 17 (Th17) cells depicted a $CD4^+$ effector T cell that produced primarily IL-17 and whose development was inhibited by the cytokines IFN-γ and IL-4 (Harrington et al., 2005; Park et al., 2005). Delving further into defining the requirements for Th17 cell development, researchers considered the cytokine TGF-β, because it had been shown previously to repress IFN-γ expression and to potently inhibit Th1 and Th2 cell differentiation through the suppression of the transcription factors T-bet and GATA3. Not surprisingly, reports demonstrating the role of TGF-β in Th17 cell development, specifically in the presence of IL-6, followed shortly thereafter. However, in a rare case where the mouse immune system appeared not to mirror its more evolved counterpart, human Th17 cells were initially shown to develop in the absence of TGF-β, requiring only IL-6 and IL-1β or IL-23 and IL-β (Acosta-Rodriguez et al., 2007; Wilson et al., 2007). Later studies refuted these observations, demonstrating a requirement for TGF-β in the development of Th17 cells from painstakingly isolated naive $CD4^+$ T cells in medium lacking serum, an oft and heretofore ignored source of varying amounts of TGF-β (Korn et al., 2009). Although the role of TGF-β in induced regulatory T (Treg) cell generation is widely accepted, considerable controversy regarding the requirement of TGF-β in directing Th17 cell development remains, and two recent works published in *Nature* (Ghoreschi et al., 2010) and here, in the current issue of *Immunity* (Gutcher et al., 2011), continue to add fuel to the fire.

Building on their previous study (Li et al., 2007) that showed T cells are the main source of TGF-β responsible for driving Th17 cell development, Gutcher et al. (2011) further dissects the T cell populations responsible for TGF-β-driven Th17 cell differentiation. The current study begins by demonstrating that all progeny of naive $CD4^+$ T cells have the potential to express TGF-β, but which of these cells are the primary source of TGF-β involved in Th17 cell development? To address this question, Gutcher et al. (2011) took advantage of the preferential expression of OX-40 in activated $CD4^+$ T cells and Treg cells and used an OX-40-driving Cre recombinase-expressing mouse strain to successfully delete *Tgfb1* in the majority of these cells, designating the resultant mice $Tgfb1^{f/n}$ *Tnfrsf4-cre.* In comparison to $Tgfb1^{f/n}$ *Cd4-cre* mice with deletion of TGF-β in all T cells (Li et al., 2007), $Tgfb1^{f/n}$ *Tnfrsf4-cre* animals developed a much less severe wasting disease and did not exhibit signs of spontaneous activation and differentiation in the periphery. Consequently, TGF-β deletion in the periphery was confined mainly to the Treg cell population, present in increased frequencies in these mice, thereby implicating Treg cell-derived TGF-β as a critical requirement of Treg cell maintenance. In the gut, though, there is a higher prevalence of activated T cells than in the periphery (Ivanov et al., 2008) and therefore the reduced percentages of gut-resident IL-17-expressing cells in $Tgfb1^{f/n}$ *Tnfrsf4-cre* mice along with their resistance to developing experimental autoimmune encephalomyelitis (EAE), resulting from decreased $IL\text{-}17^+$ cells in the central nervous system (CNS), hinted that activated T cells supply TGF-β needed for optimal Th17 cell development.

By utilizing a Foxp3-driving Cre recombinase to delete *Tgfb1* in regulatory cells (designated $Tgfb1^{f/n}$ *Foxp3-cre*), Gutcher et al. (2011) demonstrated that these animals remained healthy, without signs of inflammatory disease or spontaneous T cell activation; however, increases in frequencies and numbers of $Foxp3^+$ cells in peripheral and mesenteric lymph nodes were observed, thereby confirming Treg cells as the source of TGF-β responsible for limiting their own proliferation. Induction of EAE in $Tgfb1^{f/n}$ *Foxp3-cre* mice resulted in the development of $IL\text{-}17^+$ cells in the CNS with associated disease symptoms similar to that of wild-type mice, demonstrating that Treg cell-derived TGF-β is insufficient to direct Th17 cell development, yet a role for Treg cells in promoting Th17 cell differentiation remains plausible. Studies by Chen et al. (2011) and Pandiyan et al. (2011) in this issue of *Immunity* report that Treg cells promote Th17 cell development by consuming the Th17 cell-prohibitive cytokine, IL-2, early in Th17 cell differentiation, which simultaneously promotes their own growth and proliferation, thereby allowing for a suppressive role later in the response.

The elimination of Treg cells as the source of Th17 cell-inducing TGF-β left activated T cells as likely suspects but which members of this heterogeneous population are key? By using a TGF-β reporter mouse line, Gutcher et al. (2011) determined that in vitro polarized Th1, Th2, and Th17 cells all can express TGF-β, but it is most highly expressed in Th17 cells. Further, induction of EAE in $Rag1^{-/-}$ mice reconstituted with an equal mix of wild-type and $Tgfb1^{f/n}$ *Cd4-cre* bone marrow resulted in wild-type derived Th17 and Th1 cells in the CNS whereas substantially fewer $IL\text{-}17^+$ cells originating from the $CD4^+$ *Tgfb1*-deficient bone marrow were detected, elegantly establishing that TGF-β acts in an autocrine manner to promote Th17 cell differentiation.

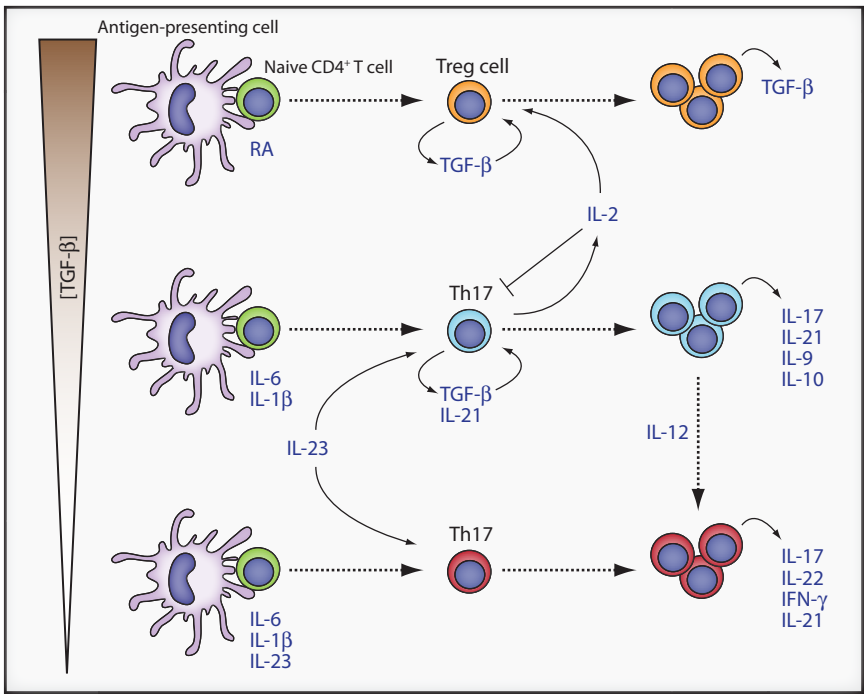

Figure 1. Proposed Th17 and Treg Cell Developmental Pathways
Naive CD4$^+$ T cells (green circles) in the presence of high concentrations of TGF-β and retinoic acid develop into regulatory T cells that produce autocrine TGF-β, instrumental in maintaining their development (orange circles). Lower concentration of TGF-β together with IL-6 and IL-1β promote the development of Th17 cells (blue circles) expressing autocrine TGF-β and IL-21, further reinforcing their own development. In the presence of IL-12, these cells can be diverted to cells expressing both IFN-γ and IL-17 (red cells). The Th17 cell-inhibiting cytokine IL-2 is also expressed but is consumed by Treg cells, promoting both Th17 cell development and Treg cell survival. In the absence of TGF-β, IL-6, IL-1β, and IL-23 together drive the development of Th17 cells with a pathogenic phenotype (red circles).

The studies of Gutcher et al. (2011) confirm and extend previous observations that T cell-derived TGF-β is required for Th17 cell differentiation, adding the unforeseen finding that Th17 cell-produced TGF-β is essential for reinforcing their own maintenance. Recently, however, Ghoreschi et al. (2010) demonstrated that Th17 cells can arise in the absence of TGF-β signaling, a seemingly contradictory finding to that of Gutcher et al. (2011). Similar to the initial reports describing the generation of human Th17 cells, Ghoreschi et al. (2010) demonstrated that IL-17$^+$ T cells can be generated from mouse naive CD4$^+$ T cells with solely IL-6, IL-23, and IL-1β, though significantly fewer IL-17$^+$ cells arise in comparison to TGF-β plus IL-6-generated cells and those cells that do develop possess a more pathogenic phenotype than do TGF-β-derived cells.

Ghoreschi et al. (2010) find that Th17 cells differentiated with TGF-β express the regulatory cytokines IL-9 and IL-10 whereas those derived without TGF-β express Th1 cell-associated molecules including IFN-γ and migrate to inflammatory sites. By using a transfer model of EAE, Ghoreschi et al. (2010) demonstrate that Th17 cells polarized without TGF-β induce a more severe disease and have greater numbers of IL-17$^+$IFN-γ$^+$ cells in the CNS compared to mice receiving TGF-β-directed Th17 cells. In normal mice, MOG peptide-induced EAE results in substantial numbers of CD4$^+$IFN-γ$^+$IL-17$^+$ T cells in the CNS, but whether these double-positive cells are derived from a TGF-β-independent pathway is unclear.

In the continued presence of TGF-β alone, Th17 cells can develop into cells expressing both IL-17 and IFN-γ, demonstrating an intrinsic property of Th17 cells to deviate to a cell that has Th1 cell-like characteristics (Lee et al., 2009). That Th17 cells can rapidly respond to IL-12 signaling by upregulating IFN-γ further emphasizes this property. It is therefore not entirely unexpected that cells generated in the absence of TGF-β express IFN-γ. However, because TGF-β is widely expressed, it is difficult to imagine a scenario where a tissue would be completely devoid of its expression, yet it is conceivable that limiting amounts in a cell's immediate vicinity would have an impact on its fate, because low concentrations of TGF-β appear to favor Th17 cell development. Ghoreschi et al. (2010) suggest that Th17 cells are derived from both TGF-β-dependent and -independent pathways, but an alternative and not mutually exclusive explanation is that the inherent plasticity of Th17 cells is amplified in conditions where high concentrations of inflammatory cytokines and limiting TGF-β tip the balance to a more aggressive phenotype (Figure 1). Nevertheless, reconciling Ghoreschi et al. (2010)'s observations with the fact that Th17 cells fail to develop in the absence of T cell-derived TGF-β in the EAE model is somewhat problematic and further complicated by the revelation that Th17 cells themselves can express TGF-β, but perhaps the ability to generate appropriate IL-1β, IL-6, and IL-23 signals in the specific contextual environment of a naive CD4$^+$ T cell is dysregulated in the *Tgfb1*$^{f/n}$ *Cd4-cre* mouse model, though this remains to be determined.

There appears to be little disagreement that Th17 cells can be generated from naive CD4$^+$ T cells in the presence of TGF-β and IL-6, and the studies of both Gutcher et al. (2011) and Ghoreschi et al. (2010) reaffirm this tenet. However, the role of autocrine TGF-β in promoting Th17 cell development at steady state requires further investigation, particularly in regards to the signals that drive a Th17 cell to produce TGF-β. The combination of IL-6, IL-23, and IL-1β was largely inefficient in this respect because the addition of neutralizing TGF-β antibodies did not effect Th17 cell development. Though considerable debate continues regarding the critical factors necessary for Th17 cell development, the varied environmental conditions in which they develop suggests a complex array of context-specific signals rather than absolute requirements. Thus, future studies focusing on the contextual and environmental requirements for Th17 cell generation both at steady state and during inflammatory conditions are needed.

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Eat Your Carrots! T Cells Are RARing to Go

Shipra Vaishnava[2] and Lora V. Hooper[1,2,*]
[1]The Howard Hughes Medical Institute
[2]Department of Immunology
The University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Boulevard, Dallas, Texas 75390, USA
*Correspondence: lora.hooper@utsouthwestern.edu
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In this issue of *Immunity*, Hall et al. (2011) show that vitamin A and its metabolites play a central role in regulating adaptive immunity by promoting the development of both inflammatory and regulatory T cell responses.

Vitamin A is an essential fat-soluble nutrient that is required for the development of many organs and tissues. It is converted into several metabolically active derivatives, including retinoic acid (RA). RA is a potent regulatory molecule that controls gene expression through RA receptors (RARs), members of the nuclear hormone receptor family that activate the transcription of specific target genes. The RA-RAR signaling machinery represents an evolutionarily ancient gene regulatory pathway, which is conserved in all vertebrates and invertebrate chordates. It appears to have evolved as a core regulatory network that governs cell fate specification during developmental patterning. In vertebrates, RA also plays important roles in the development of specific immunity, directing immunoglobulin class switching in B cells and inducing gut homing receptors on B and T lymphocytes. However, until relatively recently there was little known about how RA impacts T cell development.

In 2007, a raft of landmark papers from several laboratories revealed a critical role for RA in directing intestinal $CD4^+$ T cell development (Benson et al., 2007; Coombes et al., 2007; Denning et al., 2007; Mucida et al., 2007; Sun et al., 2007). According to these initial studies, this effect was restricted to a particular subset of $CD4^+$ T cells. $CD4^+$ T cells can develop along one of several distinct pathways that dictate the type of immune response that ensues. T helper type 1 (Th1) and Th17 cell developmental subsets are associated with aggressive proinflammatory responses to infection, whereas T regulatory (Treg) cells constitute a distinct developmental subset that is associated with immune responses that prevent or dampen inflammation. Each of the studies from 2007 showed that RA signaling triggered intestinal Treg cell development. A specific subset of intestinal dendritic cells (DCs) produced RA, which synergized with the cytokine TGF-β to induce Foxp3 in naive $CD4^+$ T cells, promoting differentiation along the Treg cell pathway (Coombes et al., 2007; Sun et al., 2007). At the same time, other studies revealed that RA inhibited Th17 cell development, leading to the idea of RA as an anti-inflammatory factor that promotes intestinal Treg cell differentiation at the expense of Th17 cell development (Elias et al., 2008; Mucida et al., 2007).

In this issue of *Immunity*, Hall et al. (2011) challenge the idea that RA is an anti-inflammatory factor that restricts T cell differentiation to the Treg cell pathway. Instead, the authors show that RA plays a much broader role in controlling $CD4^+$ T cell fate (Figure 1). To investigate the role of vitamin A and its metabolites in $CD4^+$ T cell responses during infection, Hall et al. put mice on a diet lacking vitamin A. They then provoked inflammation by using *Toxoplasma gondii*, an intracellular protozoan parasite that normally enters the body through the gastrointestinal tract, eliciting robust mucosal inflammation and a strong systemic Th1 cell response. When Hall et al. examined $CD4^+$ T cells after oral infection with *T. gondii*, they observed that mice on a vitamin A-deficient diet had a marked reduction in Th1 cell